solubility of PFPE materials in supercritical carbon dioxide has been reported. See Bunyard, W., et al., *Macromolecules* **1999**, *32*, 8224-8226. Beyond PFPEs, fluoroelastomers also can comprise fluoroolefin-based materials, including, but not limited to, copolymers of tetrafluoroethylene, hexafluoropropylene, vinylidene fluoride and alkyl vinyl ethers, often with additional cure site monomers added for crosslinking.

A PFPE microfluidic device has been previously reported by Rolland, J. et al. *JACS* **2004**, *126*, 2322-2323. The device was fabricated from a functionalized PFPE material (e.g., a PFPE dimethacrylate (MW = 4,000 g/mol)) having a viscosity of the functionalized material of approximately 800 cSt. This material was end-functionalized with a free radically polymerizable methacrylate group and UV photocured free radically with a photoinitiator. In Rolland, J. et al., *supra*, multilayer PFPE devices were generated using a specific partial UV curing technique and the adhesion was weak and generally not strong enough for a wide range of applications. Further, the adhesion technique described by Rolland, J. et al. did not provide for adhesion to other substrates such as glass.

The presently disclosed subject matter describes the use of fluoroelastomers, especially a functional perfluoropolyether as a material for fabricating solvent-resistant micro-and nano-scale structures, such as a microfluidic device. The use of fluoroelastomers and functional perfluoropolyethers in particular as materials for fabricating a microfluidic device addresses the problems associated with swelling in organic solvents exhibited by microfluidic devices made from other polymeric materials, such as PDMS. Accordingly, PFPE-based microfluidic devices can be used to control the flow of a small volume of a fluid, such as an organic solvent, and to perform micro- and nano-scale chemical reactions that are not amenable to other polymeric microfluidic devices.

SUMMARY

The presently disclosed subject matter provides functional perfluoropolyether (PFPE) materials for use in fabricating microfluidic devices. In some embodiments, the presently disclosed subject matter provides a

PFPE material and the curing reaction is taken to completion, thereby forming a permanent bond between the PFPE layers.

Further, the partially cured PFPE network can be contacted with a layer or substrate comprising another polymeric material, such as poly(dimethylsiloxane) or another polymer, and then thermally cured so that the PFPE network adheres to the other polymeric material. Additionally, the partially cured PFPE network can be contacted with a solid substrate, such as glass, quartz, or silicon, and then bonded to the substrate through the use of a silane coupling agent.

III.A. Method of Forming a Patterned Layer of an Elastomeric Material

In some embodiments, the presently disclosed subject matter provides a method of forming a patterned layer of an elastomeric material. The presently disclosed method is suitable for use with the perfluoropolyether material described in Sections II.A. and II.B., and the fluoroolefin-based materials described in Section II.C. An advantage of using a higher viscosity PFPE material allows, among other things, for a higher molecular weight between cross links. A higher molecular weight between cross links can improve the elastomeric properties of the material, which can prevent among other things, cracks from forming. Referring now to Figures 1A-1C, a schematic representation of an embodiment of the presently disclosed subject matter is shown. A substrate **100** having a patterned surface **102** comprising a raised protrusion **104** is depicted. Accordingly, the patterned surface **102** of the substrate **100** comprises at least one raised protrusion **104**, which forms the shape of a pattern. In some embodiments, patterned surface **102** of substrate **100** comprises a plurality of raised protrusions **104** which form a complex pattern.

As best seen in Figure 1B, a liquid precursor material **106** is disposed on patterned surface **102** of substrate **100**. As shown in Figure 1B, the liquid precursor material **106** is treated with a treating process T_r. Upon the treating of liquid precursor material **106**, a patterned layer **108** of an elastomeric material (as shown in Figure 1C) is formed.

As shown in Figure 1C, the patterned layer **108** of the elastomeric

of PFPE material to a substrate is illustrated in Figures 3A-3C. Referring now to Figure 3A, a substrate **300** is provided, wherein, in some embodiments, substrate **300** is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. Substrate **300** is treated by treating process T_{r1}. In some embodiments, treating process T_{r1} comprises treating the substrate with a base/alcohol mixture, e.g., KOH/isopropanol, to impart a hydroxyl functionality to substrate **300**.

Referring now to Figure 3B, functionalized substrate **300** is reacted with a silane coupling agent, e.g., R-$SiCl_3$ or R-$Si(OR_1)_3$, wherein R and R_1 represent a functional group as described herein to form a silanized substrate **300**. In some embodiments, the silane coupling agent is selected from the group consisting of a monohalosilane, a dihalosilane, a trihalosilane, a monoalkoxysilane, a dialkoxysilane, and a trialkoxysilane; and wherein the monohalosilane, dihalosilane, trihalosilane, monoalkoxysilane, dialkoxysilane, and trialkoxysilane are functionalized with a moieties selected from the group consisting of an amine, a methacrylate, an acrylate, a styrenic, an epoxy, an isocyanate, a halogen, an alcohol, a benzophenone derivative, a maleimide, a carboxylic acid, an ester, an acid chloride, and an olefin.

Referring now to Figure 3C, silanized substrate **300** is contacted with a patterned layer of partially cured PFPE material **302** and treated by treating process T_{r2} to form a permanent bond between patterned layer of PFPE material **302** and substrate **300**.

In some embodiments, a partial free radical cure is used to adhere a PFPE layer to a second polymeric material, such as a poly(dimethyl siloxane) (PDMS) material, a polyurethane material, a silicone-containing polyurethane material, and a PFPE-PDMS block copolymer material. In some embodiments, the second polymeric material comprises a functionalized polymeric material. In some embodiments, the second polymeric material is encapped with a polymerizable group. In some embodiments, the polymerizable group is selected from the group consisting of an acrylate, a styrene, and a methacrylate. Further, in some embodiments, the second polymeric material is treated with a plasma and a silane coupling agent to

V.A. Method of Attaching a Functional Group to a PFPE Network

In some embodiments, PFPE networks comprising excess functionality are used to functionalize the interior surface of a microfluidic channel or the surface of a microtiter well. In some embodiments, the interior surface of a microfluidic channel or the surface of a microtiter well is functionalized by attaching a functional moiety selected from the group consisting of a protein, an oligonucleotide, a drug, a ligand, a catalyst, a dye, a sensor, an analyte, and a charged species capable of changing the wettability of the channel.

In some embodiments, latent functionalities are introduced into the fully cured PFPE network. In some embodiments, latent methacrylate groups are present at the surface of the PFPE network that has been free radically cured either photochemically or thermally. Multiple layers of fully cured PFPE are then contacted with the functionalized surface of the PFPE network, forming a seal, and reacted, by heat, for example, to allow the latent functionalities to react and form a permanent bond between the layers.

In some embodiments, the latent functional groups react photochemically with one another at a wavelength different from that used to cure PFPE precursors. In some embodiments, this method is used to adhere fully cured layers to a substrate. In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. In some embodiments, the substrate is treated with a silane coupling agent complimentary to the latent functional groups.

In some embodiments, such latent functionalities are used to adhere a fully cured PFPE network to a second polymeric material, such as a poly(dimethylsiloxane) (PDMS) material. In some embodiments, the PDMS material comprises a functionalized PDMS material. In some embodiments, the PDMS material is treated with a plasma and a silane coupling agent to introduce the desired functionality. In some embodiments, the PDMS material is encapped with a polymerizable group. In some embodiments, the polymerizable group is selected from the group consisting of an acrylate, a styrene, and a methacrylate.

nm UV light, which can strip a fluorine atom off of the back bone and form a chemical bond to the substrate as described by Vurens, G., et al. *Langmuir* **1992,** *8*, 1165-1169. Thus, in some embodiments, the PFPE layer is covalently bonded to the solid substrate by radical coupling following abstraction of a fluorine atom.

VIII. Adhesion of a Microscale or a Nanoscale Device to a Substrate through an Encasing Polymer

In some embodiments, a microscale device, a nanoscale device, or combinations thereof is adhered to a substrate by placing the fully cured device in conformal contact on the substrate and pouring an "encasing polymer" over the entire device. In some embodiments, the encasing polymer is selected from the group consisting of a liquid epoxy precursor and a polyurethane. The encasing polymer is then solidified by curing or other methods. The encasement serves to bind the layers together mechanically and to bind the layers to the substrate.

In some embodiments, the microscale device, the nanoscale device, or combinations thereof comprises one of a perfluoropolyether material as described in Section II.A and Section II.B. hereinabove and a fluoroolefin-based material as described in Section II.C. hereinabove.

In some embodiments, the substrate is selected from the group consisting of a glass material, a quartz material, a silicon material, a fused silica material, and a plastic material. Further, in some embodiments, the substrate comprises a second polymeric material, such as poly(dimethylsiloxane) (PDMS), or another polymer. In some embodiments, the second polymeric material comprises an elastomer other than PDMS, such as Kratons, buna rubber, natural rubber, a fluorelastomer, chloroprene, butyl rubber, nitrile rubber, polyurethane, or a thermoplastic elastomer. In some embodiments, the second polymeric material comprises a rigid thermoplastic material, including but not limited to: polystyrene, poly(methyl methacrylate), a polyester, such as poly(ethylene terephthalate), a polycarbonate, a polyimide, a polyamide, a polyvinylchloride, a polyolefin, a poly(ketone), a poly(ether ether ketone), and a poly(ether sulfone). In some

individual molecules, such as nucleic acids, polypeptides or other organic molecules, or larger cell components like organelles. The method can sort for cell viability or other cellular expression functions.

Amplification, separation, sequencing, and identification of nucleic acids and proteins are common microfluidic device applications. For example, U.S. Patent No. 5,939,291 to Loewy et al. illustrates a microfluidic device that uses electrostatic techniques to perform isothermal nucleic acid amplification. The device can be used in conjunction with a number of common amplification reaction strategies, including PCR (polymerase chain reaction), LCR (ligase chain reaction), SDA (strand displacement amplification), NASBA (nucleic acid sequence-based amplification), and TMA (transcription-mediated amplification). U.S. Patent No. 5,993,611 to Moroney et al. describes a device that uses capacitive charging to analyze, amplify or otherwise manipulate nucleic acids. Devices have been designed that sort DNA by size, analyzing restriction fragment length polymorphism (see U.S. Patent No. 6,833,242 to Quake et al.). The devices also can have particular use in forensic applications, such as DNA fingerprinting. U.S. Patent No. 6,447,724 to Jensen et al. describes microfluidics that identify components of a mixture based on the different fluorescent lifetimes of the labels attached to members of the mixture. Such a device could be used to analyze sequencing reactions of nucleic acids, proteins or oligosaccharides or to inspect or interrogate members of a combinatorial library of organic molecules.

Other microfluidic devices directed toward specific protein applications include a device that promotes protein crystal growth in microfluidic channels (see U.S. Patent No. 6,409832, to Weigl et al.). In the device, protein sample and solvent are directed to a channel with laminar flow characteristics that form diffusion zones, which provide well-defined crystallization. U.S. Published Patent Application No. 2004/0121449 to Pugia et al. illustrates a device that can separate red blood cells from plasma using minimal centrifugal force on sample sizes as small as 5 microliters. The device could be particularly useful in clinical diagnostics and also could be used to separate any particulate matter from a liquid.

As partly described hereinabove, microfluidic devices have been

gas separation membrane comprises a stand-alone film. In some embodiments, the gas separation membrane comprises a composite film.

In some embodiments, the gas separation membrane comprises a co-monomer. In some embodiments, the co-monomer regulates the permeability properties of the gas separation membrane. Further, the mechanical strength and durability of such membranes can be finely tuned by adding composite fillers, such as silica particles and others, to the membrane. Accordingly, in some embodiments, the membrane further comprises a composite filler. In some embodiments, the composite filler comprises silica particles.

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

General Considerations

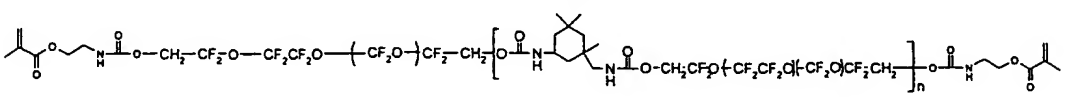
A PFPE microfluidic device has been previously reported by Rolland, J. et al. *JACS* **2004**, *126*, 2322-2323, which is incorporated herein by reference in its entirety. The specific PFPE material disclosed in Rolland, J., et al., was not chain extended and therefore did not possess the multiple hydrogen bonds that are present when PFPEs are chain extended with a diisocyanate linker. Nor did the material possess the higher molecular weights between crosslinks that are needed to improve mechanical properties such as modulus and tear strength which are critical to a variety of microfluidics applications. Furthermore, this material was not functionalized to incorporate various moieties, such as a charged species, a biopolymer, or a catalyst.

Herein is described a variety of methods to address these issues. Included in these improvements are methods which describe chain extension,

improved adhesion to multiple PFPE layers and to other substrates such as glass, silicon, quartz, and other polymers as well as the ability to incorporate functional monomers capable of changing wetting properties or of attaching catalysts, biomolecules or other species. Also described are improved methods of curing PFPE elastomers which involve thermal free radical cures, two-component curing chemistries, and photocuring using photoacid generators.

Example 1

A liquid PFPE precursor having the structure shown below (where n = 2) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately, a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Thirdly, a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The slide is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the fully cured PFPE smooth layer on the glass slide and allowed to heat at 120 °C for 15 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science*. **2000,** *288*, 113-6.



Example 29

Fabrication of a PFPE Microfluidic Device using Sacrificial Channels

A smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE dimethacrylate precursor across a glass slide. The slide is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. A scaffold composed of poly(lactic acid) in the shape of channels is laid on top of the flat, smooth layer of PFPE. A liquid PFPE dimethacrylate precursor is with 1 wt% of a free radical photoinitiator and poured over the scaffold. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The apparatus is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The device is then heated at 150 °C for 24 hours to degrade the poly(lactic acid) thus revealing voids left in the shape of channels.

Example 30

Adhesion of a PFPE Device to Glass using 185-nm Light

A liquid PFPE dimethacrylate precursor is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes

are punched using a luer stub. The bonded layers are then placed on a clean, glass slide in such a way that it forms as seal. The apparatus is exposed to 185 nm UV light for 20 minutes, forming a permanent bond between the device and the glass. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science* **2000,** *288*, 113-6.

Example 31

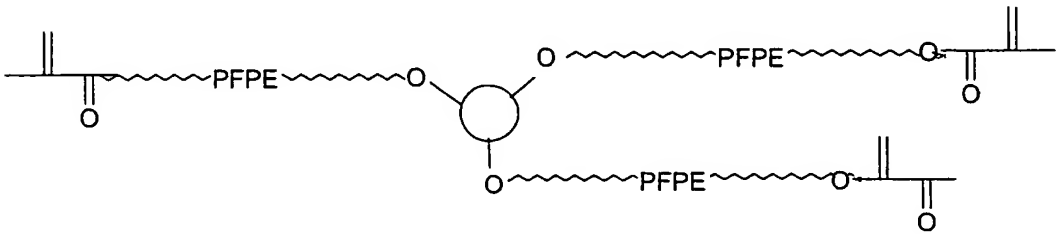
"Epoxy Casing" Method to Encapsulate Devices

A liquid PFPE dimethacrylate precursor is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on a clean, glass slide in such a way that it forms as seal. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science* **2000,** *288*, 113-6. The entire apparatus is then encased in a liquid epoxy precursor which is poured over the device allowed to cure. The casing serves to mechanically bind the device the substrate.

Example 32

Fabrication of a PFPE Device from a Three-Armed PFPE Precursor

A liquid PFPE precursor having the structure shown below (where the circle represents a linking molecule) is blended with 1 wt% of a free radical photoinitiator and poured over a microfluidics master containing 100-μm features in the shape of channels. A PDMS mold is used to contain the liquid in the desired area to a thickness of about 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Separately a second master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE precursor over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. Thirdly a smooth, flat PFPE layer is generated by drawing a doctor's blade across a small drop of the liquid PFPE precursor across a glass slide. The slide is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The thicker layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The layer is then placed on top of the 20-μm thick layer and aligned in the desired area to form a seal. The layers are then placed in an oven and allowed to heat at 120 °C for 2 hours. The thin layer is then trimmed and the adhered layers are lifted from the master. Fluid inlet holes and outlet holes are punched using a luer stub. The bonded layers are then placed on the fully cured PFPE smooth layer on the glass slide and allowed to heat at 120 °C for 15 hours. Small needles can then be placed in the inlets to introduce fluids and to actuate membrane valves as reported by Unger, M. et al. *Science* **2000**, *288*, 113-6.



Example 33

Photocured PFPE/PDMS Hybrid

A master containing 100-μm features in the shape of channels is spin coated with a small drop of the liquid PFPE dimethacrylate precursor containing photoinitiator over top of it at 3700 rpm for 1 minute to a thickness of about 20 μm. A PDMS dimethacrylate containing photoinitiator is then poured over top of the thin PFPE layer to a thickness of 3 mm. The wafer is then placed in a UV chamber and exposed to UV light (λ = 365) for 10 minutes under a nitrogen purge. The layer is then removed, trimmed, and inlet holes are punched through it using a luer stub. The hybrid device is then placed on a glass slide and a seal is formed. Small needles can then be placed in the inlets to introduce fluids.

It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.